Standard Operating Procedure for Total Kjeldahl Nitrogen (Lachat Method)

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Revision 2

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1.0 Scope and Application

1.1 This method covers the determination of Total Kjeldahl Nitrogen (TKN) in lake and rain water.

1.2 The approximate working range is 0.10 to 2.50 mg-N/L. The method detection limit is 0.10 mg-N/L.

2.0 Summary

2.1 Samples are digested in sulfuric acid in the presence of a mercuric oxide catalyst. The Kjeldahl nitrogen present is converted to ammonium cation. Potassium sulfate helps speed the conversion to ammonium.

2.2 After injection onto the manifold, the samples pH is raised to a known, basic pH with a concentrated buffer. This neutralization converts the ammonium to ammonia. The ammonia is heated with salicylate and hypochlorite to produce a blue color which is proportional to the ammonia concentration. The color is intensified by adding sodium nitroprusside. The presence of tartrate in the buffer prevents precipitation of calcium and magnesium.

3.0 Sample Handling and Preservation

3.1 Samples are collected in new or acid-washed glass or plastic containers.

3.2 Samples are preserved by addition of 1 mL of H_2SO_4 per liter of samples. Store at room temperature.

4.0 Interferences

4.1 Samples must not consume more than half of the sulfuric acid during digestion. The buffer will accommodate a range of 2-4% (v/v) H_2SO_4 in the diluted digested sample with no change in signal intensity.

4.2 Incomplete digestion, evident by dark particles in digested samples may cause low results. When this occurs, the original sample must be diluted and redigested.

5.0 Apparatus

5.1 Digestion tubes: 1"x 8" heavy-walled pyrex tubes.

5.2 Block Digester.

5.3 Adjustable pipets with disposable tips capable of delivering 10 mL and 2 mL volumes.

5.4 13 x 100 mm disposable test tubes.

5.5 Lachat QuikChem AE

5.5.1 XYZ Sampler

5.5.2 TKN Manifold (Lachat Manifold #10-107-06-2-E)

5.5.3 Printer

6.0 Reagents and Standards

6.1 All reagents should be stored in the appropriate bottles and labeled with the following information:

Identity:	(Buffer)
Date:	(mm/dd/yy)
Initials of Preparer:	(M.S.)

All standards will be stored in appropriate bottles and labeled as above with the following also included:

Concentration:	(100 mg-N/L)

6.2 Use deionized water for all solutions.

6.3 Digestion Solution: Add 4 mL of concentrated H_2SO_4 to 21 mL water. Dissolve 2.0 g mercuric oxide (HgO) in the solution. Set this aside.

In a 1 L volumetric flask carefully add 200 mL of concentrated H_2SO_4 to about 500 mL water. While this solution is still hot, dissolve 134 g of potassium sulfate (K_2SO_4) in it. Add the HgO solution. Cool and dilute. Store at room temperature. Do not allow salt to precipitate. If precipitation does occur, put reagent bottle in warm water bath for about 30 minutes. Stir on stir plate until precipitation is no longer evident.

6.4 Buffer: In a 1 L volumetric flask dissolve 65 g sodium hydroxide (NaOH), 20.0 g disodium EDTA (ethylenediaminetetraacetic acid disodium salt), and 35.0 g sodium phosphate dibasic heptahydrate ($Na_2HPO_4 \cdot 7H_2O$) in about 900 mL water. Dilute to the mark and invert to mix. De-gas with helium.

6.5 Salicylate - Nitroprusside Reagent: In a 1 L volumetric flask dissolve 150.0 g sodium salicylate [salicylic acid sodium salt, $C_6H_4(OH)(COO)Na$], and 1.00 g sodium nitroprusside [sodium nitroferricyanide dihydrate, $Na_2Fe(CN)_5NO \cdot 2H_2O$] in about 800 mL water. Dilute to the mark and invert to mix. Store in a dark bottle and prepare fresh monthly. De-gas with helium.

6.6 Hypochlorite Solution: In a 500 mL volumetric flask, dilute 30 mL Regular Clorox Bleach (5.25% sodium hypochlorite) to the mark with water. Invert to mix. De-gas with helium.

6.7 Diluent: **Note:** Diluent is prepared for use in the auto-dilutor to dilute off-scale samples. This reagent is not used on-line. In a 1 L volumetric flask containing approximately 600 mL water, add 240 mL Digestion Solution (6.3). Dilute to the mark and invert to mix.

6.8 Preparation of Standards

6.8.1 Stock 100 mg-N/L Nitrogen Standard: In a 1 L volumetric flask, dissolve 1.050 g dried L-(+)-glutamic acid in 500 mL water. Add 1 mL of concentrated H_2SO_4 and dilute to the mark.

6.8.2 Working Standards: Prepare standards over the range of analysis. For the working range of 0-2.50 mg-N/L, the following standards may be used:

mL Stock Solution(6.8.1) diluted to 1 L	Concentration mg-N/L
0.00	0.00
1.00	0.10
2.50	0.25
5.00	0.50
7.50	0.75
10.00	1.00
25.00	2.50

Note: Use volumetric flasks. Preserve the working standards by addition of 1 mL of concentrated H_2SO_4.

6.8.3 Stock 227 mg-N/L Nitrogen Control Standard: In a 1L volumetric flask dissolve 1.3845 g of adenosine-5-monophosphoric acid disodium salt in 500 mL water. Add 1 mL of conc. H_2SO_4 and dilute to the mark.

6.8.4 Working Control Standards: The following concentrations are typical:

mL Stock Control Standard (6.8.3) diluted to 1 L	Concentration mg-N/L
CH 5.0	1.15
CL 2.0	0.45

Note: Use volumetric flasks. Preserve the control standards by addition of 1 mL of concentrated H_2SO_4.

7.0 Procedure

7.1 Digestion

7.1.1 Rinse all glassware once with 1:1 HCl, and then three times with deionized water. Do not use commercial detergents.

7.1.2 Using an automatic pipet with disposable tips, withdraw a 10 mL aliquot of sample. Discard this first portion. Withdraw another 10 mL aliquot and transfer to a digestion tube.

7.1.3 Add 2.0 mL of digestion solution (6.3) and several (two to three) boiling chips.

7.1.4 Prepare all samples, calibration standards, blanks, control standards, spikes, and duplicates in the same manner.

7.1.5 Place the rack of tubes in a pre-heated block digester at 200°C for 60 minutes. Be sure to place the end plates in position on the racks so heating occurs evenly.

7.1.6 Transfer the rack to a pre-heated high temperature block. Heat at 370°C for 30 minutes.

7.1.7 Remove the tubes from the block and allow to cool for about 15 minutes.

7.1.8 Add a 10 mL aliquot of deionized water to each tube. Mix the samples well using a Vortex mixer. Transfer to 13 x 100 mm test tubes for analysis. Samples may also be covered with aluminum foil and held for later analysis.

7.2 Analysis of Digested Samples

7.2.1 Allow at least 15 minutes for the heating unit to warm up to 60°C.

7.2.2 If the salicylate reagent is merged with a sample containing sulfuric acid in the absence of the buffer solution, the salicylate reagent will precipitate. To prevent this, prime the system by first placing the buffer transmission line in the buffer. Pump until the air bubble introduced during the transfer reaches the "T" fitting on the manifold. Then place all other lines in the proper containers. If precipitation does occur, all teflon tubing should be replaced.

7.2.3 It is very important that all reagents be purged thoroughly with helium before beginning analysis. Usually two to three minutes will suffice for each reagent.

7.2.4 Follow the Lachat Procedural SOP for the remainder of the analysis.

7.2.5 The diluent in the auto dilutor is reagent 6.7 not deionized water.

7.2.6 In normal operation, the digested blank will result in a slight peak. This is due to the acid in the digest and is present in every injection. Since this blank is constant for all samples and standards it will not effect data quality.

8.0 Calculations

The computer yields results directly in mg-N/L.

9.0 Quality Control

9.1 The minimum acceptable correlation coefficient (r) for TKN calibration curve is 0.995.

9.2 The following items are required with the minimum frequency indicated:

	Audit	Type	Frequency	Limits
Rain:				
	CH	Method	Beg, End, 1/40 Samp.	1.15 ± 0.15
	CL	Method	Beg, End, 1/40 Samp.	0.45 ± 0.12
	Reagent Blank(LB)	Method	Beg, End, 1/40 Samp.	0.00 ± 0.10
	Lab Blank(RB)	Method	Beg, End, 1/40 Samp.	0.00 ± 0.10
	Duplicate(LD)	Method	1/40 Samp.	$\triangle \leq 0.10$
	Spike(LSF)	Method	1/40 Samp.	100 ± 24
Lake:				
	CH	Method	Beg, End, 1/40 Samp.	1.15 ± 0.15
	CL	Method	Beg, End, 1/40 Samp.	0.45 ± 0.12
	Reagent Blank(RB)	Method	Beg, End, 1/40 Samp.	0.00 ± 0.10

10.0 Waste Disposal

Effluent from this channel is acidic and should be disposed of in a yellow labeled waste container.

11.0 Preventive Maintenance

Required maintenance is described in the Lachat Procedural SOP.

12.0 Troubleshooting

12.1 If the baseline drifts, peaks are too wide, or problems with precision arise, clean the manifold by the following procedure:

12.1.1. Place all reagent transmission lines in water and pump to clear reagents (two to five minutes).

12.1.2. Place reagent lines and carrier in 1 M HCl (one volume of HCl added to 11 volumes of water) and pump for several minutes.

12.1.3. Place all transmission lines in water and pump for several minutes.

12.1.4. Resume pumping reagents.

13.0 References

13.1 Lachat Instruments, Method Number 10-107-06-2-E, Total Kjeldahl Nitrogen in waters, Revision Date July 1993.

13.2 Lachat QuikChem AE Operating Manual.

13.3 GLAS Standard Operating Procedure, Total Kjeldahl Nitrogen. July 1992.

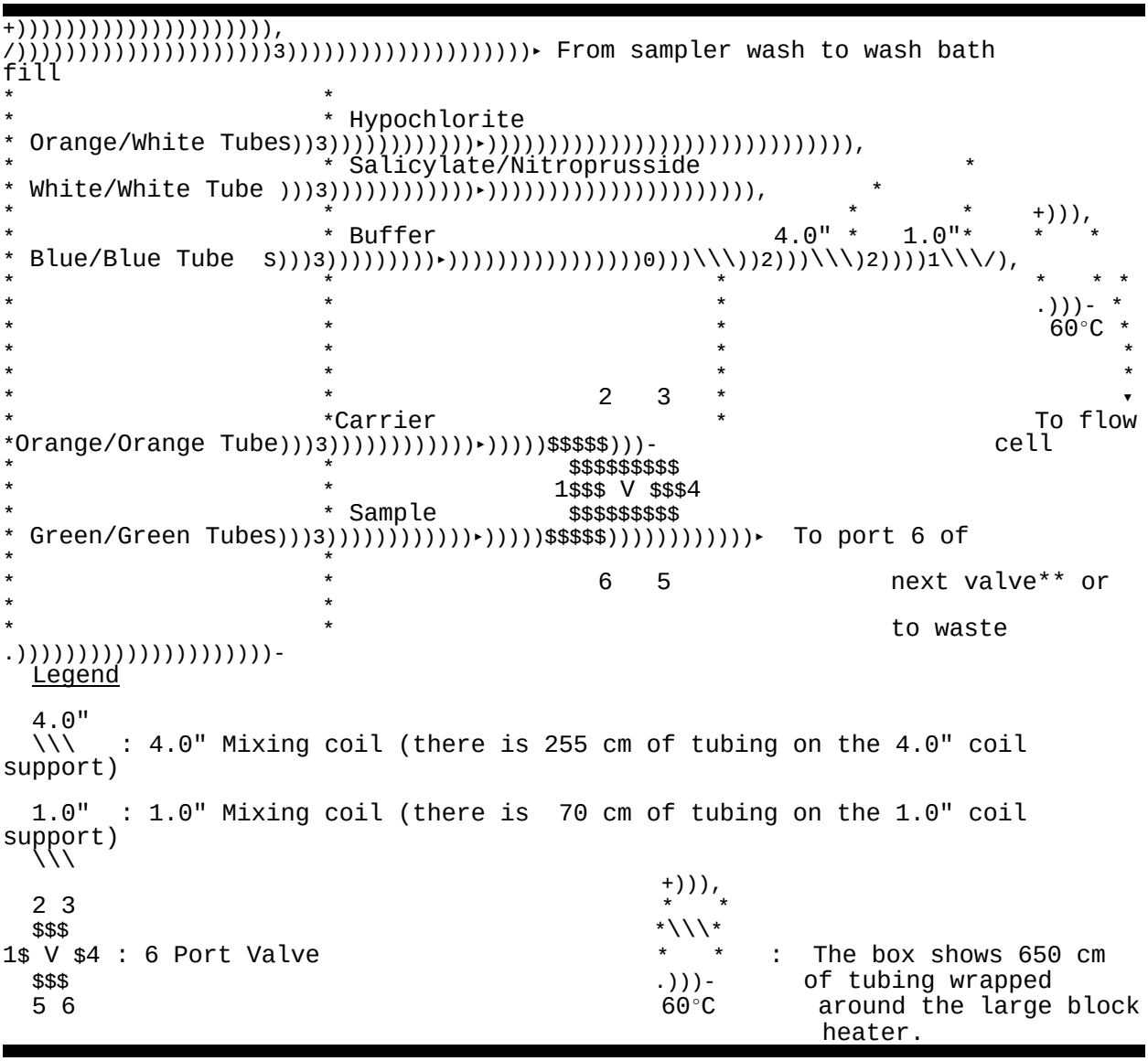

Figure 1. TKN Analytical Manifold (Lake and Rain Water)

Comments

a. Filter used is 660 nm.
b. Sample loop length is 25 cm.
c. All manifold tubing is 0.8mm (0.032")ID. This relates to a flow of 5.2 µL/cm.
d. The Carrier is helium degassed DI Water.
e. Timing: Cycle period is 49 seconds. Inject to start of peak is 47 seconds.

** If more than one channel is being used.

NUTRIENTS SECTION QUALITY CONTROL SHEET

ANALYTE: TKN **PROGRAM:** LIMNOLOGY **DATA SET:**_____

DATE	SAMPLE		CHECK STANDARD AUDIT		BLANK AUDIT
	FROM	TO	CH	CL	REAGENT BLANK (LB)
			(1.00 to 1.30)	(0.33 to 0.57)	(-0.10 to 0.10)

COMMENTS:_____

_____

ANALYST:_____DATE:____/____/____ TEAM LEADER:_____DATE:____/____/____